Proprietary

Colorimetric Determination of Glycine

Quality Control Analytical Test Method # 42

Reagents:

Methyl Cellosolve - Reagent grade

Citrate Buffer - Dissolve 21.0 grams of Citric Acid Monohydrate in 200ml. of deionized water.
Add 200 ml. of N/1 NaOH and adjust to pH 5.0 with N/1 HCl or N/1 NaOH as needed.
Dilute to volume with water in a 500 ml. vol. flask.
Recheck pH and adjust as necessary.
Refrigerate when not in use.

Potassium Cyanide - (0.1 M)
Solution #1 is prepared by adding 162.8 mg. (.1628 g) KCN crystal to a 250 ml. vol. flask and diluting to volume with deionized water.
Solution #2 (0.1M) is prepared by pipeting 5 ml. of Solution #1 into a 250 ml. vol. flask and dilute to volume with methyl cellosolve.

Ethanol - 60/40 v/v 95% Ethanol and deionized water.

Ninhydrin - 5% solution in methyl cellosolve.

KCN/Ninhydrin Reagent - Using a 100 ml. graduated cylinder, add 50 m. of KCN solution #2.
Add 10 m. of ninhydrin solution.
Reagent is stable for one week.
(Fresh solution must be at least 3 hours old before use.)
Refrigerate when not in use.

Standard Solution of Glycine - Dissolve 4 grams of Glycine in deionized water. Dilute to volume in a 1 liter vol. flask. This is the stock solution and can be stored for six months. Take 10 mls. of this solution and dilute in a 1 liter vol. flask with deionized water. Each ml. of this solution contains 40 ug of Glycine. Second solution is prepared fresh daily.

Sample Preparation

Weigh to nearest 0.1 mg enough sample to contain approximately 0.05 grams glycine. Dissolve in distilled water. (See attached chart for sample weights.) Dilute to volume in a 1 liter vol. flask. To remove insoluble material, allow the solution to sit in the liter vol. flask with occasional slight rotation of the flask. Insolubles that rise can be removed by rapidly upending the flask with the stopper removed.

Equipment Required

UV-Visible light spectrophotometer, pyrex cells, 1.0 cm.
50 ml. pyrex test tubes
Class A pipets of appropriate volumes
Bunsen burner
Water bath

Test Procedure

Pipet 1 ml. of deionized water into one test tube to be used as a blank.
Pipet 1.0, 1.5 mls. of the standard Glycine solution to separate test tubes.
Pipet 1 ml. of a sample containing 45-55ug of Glycine into a test tube.
Add 0.5 ml. of Citrate buffer to each tube.
Add 2.0 ml. of KCN/Ninhydrin reagent to each tube.*
Mix and place tubes in a steam bath for 15 minutes. A deep purple color will develop in the standard and sample test tubes.
Dilute each vol. test tube to volume with the 60% Ethanol.
Determine the Absorbance of each standard and sample against the blank of the spectrophotometer at 570 mu.

Operation of UV-Visible Spectrophotometer

1. Turn on the power switch.
2. The "INITIALIZATION" screen comes up.
3. Leave the instrument intact for about 4-5 minutes, after turning on the power switch.
4. The screen displays "INITIALIZATION"
5. If all the items are normal, the instrument is set for work.
6. Press "Parameter"
7. The screen comes up, select "Abs", press "0", then "Enter".
8. The screen comes up, select "Abs", press "2", then "Enter".
9. Press "Parameter".
10. When the screen comes up select wavelength range, press "b", then "Enter".
11. Type highest wavelength 620nm, then press "Enter".
12. Type lowest wavelength 520 nm, then press "Enter".
13. Fill up both cuvettes with blank and place in instrument.
14. Press "Baseline"; Baseline correction of the whole wavelengths takes about 3 or 4 minutes.
15. After "Baseline" is corrected (zero) abs ~ 0
16. Press "Go to λ"
17. Select "wavelength 570 nm, then press "Enter".
18. Press "Function".
19. Select "Auto Calc", press "2", then "Enter".
20. Select "Yes" for calibration, press 1, then "Enter".
21. Input concentration values of all the standard liquids with the keys. Maximum 20 standard liquids may be used.
22. Input concentration for the standard liquids of the Glycine solutions:
    Type: 40 ppm, then press "Enter".
    60 ppm, then press "Enter".
23. Select "Enter"
24. Measure absorbance of the standard liquids in the order of the selection, when the screen comes up.
25. Fill up one cuvette with standard liquid 40 ppm, press "start". Then 60ppm, press "start".
26. When measurement of all the standard liquids is completed the computer calculates and prints out the results.
27. Input samples for measure of absorbance. Fill up the cuvette in the order inputted and press start after each sample.
28. The results of calculation based on absorbance and concentration are displayed on the screen and printed out on the recorder one after another.
29. When the calculation program is ended, press "Enter".
30. Turn off the power switch

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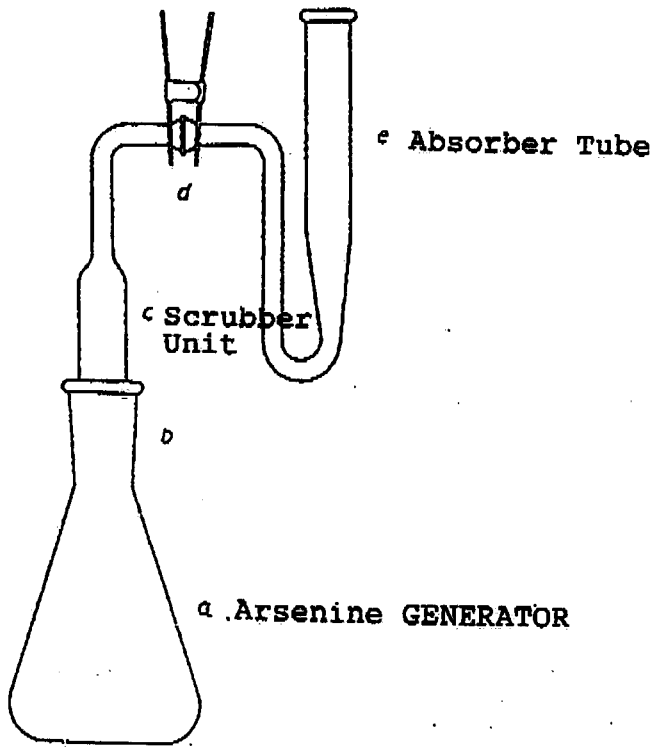
WESTWOOD CHEMICAL CORPORATION

Title	Code	Supersedes	Pg. 1/3
ANALYTICAL METHODS	ATP003		
Subject ARSENIC (211) METHOD I	Issue 2	Approved By RA	Date 02/28/80

Reviewed 12/10/01
Diane Reid

APPARATUS

The apparatus (see illustration) consists of an arsenic generator (a) fitted with a scrubber unit (c) and an absorber tube (e) with standard-taper or ground glass ball-and-socket joints (b and d) between the units. However, any other suitable apparatus, embodying the principle of the assembly described and illustrated, may be used.



Arsenic Test Apparatus

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WESTWOOD CHEMICAL CORPORATION

Title	Code	Supersedes	Pg. 2/3
ANALYTICAL METHODS	ATP003		
Subject	Issue	Approved By	Date
ARSENIC (211) METHOD I	2	RG/UH	02/28/80

Reviewed 12/10/01 Diane Reed

ARSENIC TRIOXIDE STOCK SOLUTION

Dissolve 132.0 mg of arsenic trioxide, previously dried at 105° for 1 hour and accurately weighed, in 5 L of sodium hydroxide solution (1 in 5) in a 1000 mL volumetric flask. Neutralize the solution with 2 N sulfuric acid, add 10 mL more of 2 N sulfuric acid, then add recently boiled and cooled water to volume, and mix.

STANDARD ARSENIC SOLUTION

Transfer 10.0 mL of Arsenic Trioxide Stock Solution to a 1000 mL volumetric flask, add 10 mL of 2 N sulfuric acid, then add recently boiled and cooled water to volume, and mix. Each mL of Standard Arsenic Solution contains the equivalent of 1 ug of arsenic (As). Keep this solution in an all-glass container, and use within 3 days.

STANDARD PREPARATION

Pipet 3.0 mL of Standard Arsenic Solution into a generator flask, and dilute with water to 35 mL.

TEST PREPARATION

Unless otherwise directed in the individual monograph, transfer to the generator flask the quantity, in g, of the test substance calculated by the formula:

$$3.0/L,$$

in which L is the arsenic limit in ppm, dissolve in water, and dilute with water to 35 mL.

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WESTWOOD CHEMICAL CORPORATION

Title	Code	Supersedes	Pg. 3 / 3
ANALYTICAL METHODS	ATP003		
Subject	Issue	Approved By	Date
ARSENIC (211 METHOD I	2		02/28/80

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PROCEDURE

Treat the Standard Preparation and the Test Preparation similarly as follows. Add 20 mL of 7 N sulfuric acid, 2 mL of potassium iodide TS, 0.5 mL of stronger acid stannous chloride TS, and 1 mL of isopropyl alcohol, and mix. Allow to stand at room temperature for 30 minutes. Pack the scrubber tube (c) with two pledgets of cotton that have been soaked in saturated lead acetate solution, freed from excess solution by expression, and dried in vacuum at room temperature, leaving a 2 mm space between the two pledgets. Lubricate the joints (b and d) with a suitable stopcock grease designed for use with organic solvents, and connect the scrubber unit to the absorber tube (e). Transfer 3.0 mL of silver diethyldithiocarbamate TS to the absorber tube. Add 3.0 g of granular zinc (No. 20 mesh) to the mixture in the flask, immediately connect the assembled scrubber unit, place the generator flask (a) in a water bath maintained at a temperature of 25 ± 3°, and allow the evolution of hydrogen and the color development to proceed for 45 minutes, swirling the flask gently at 10 minute intervals. Disconnect the absorber tube from the generator and scrubber units, and transfer the absorbing solution to a 1 cm absorption cell. Any red color produced by the Test Preparation does not exceed that produced by the Standard Preparation. If necessary or desirable, determine the absorbance at the wavelength of maximum absorbance between 535 and 540 nm, with a suitable spectrophotometer or colorimeter, using silver diethyldithiocarbamate TS as the blank.